Original Article

Phenotypic detection of Metallo Beta Lactamase Producing Pseudomonas aeruginosa among Clinical Isolates from the intensive care unit of a tertiary care centre

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ABSTRACT

Background: Pseudomonas aeruginosa is an opportunistic pathogenic bacterium responsible for both, acute and chronic infection, causing serious infections in patients who are mechanically ventilated individuals, who are immunocompromised, and patients with malignancies or HIV infection. **Objectives**: The phenotypic screening of metallo beta-lactamases (MBL) in strains of Pseudomonas aeruginosa resistant to imipenem. **Material and Methods**: The study was carried out in the department of Microbiology, in a medical hospital, Bareilly, over a period 18 months from January 2013 to June 2014. A total of 103 Pseudomonas aeruginosa isolated from sputum, broncho alveolar lavage (BAL), urine, pus and blood from critically ill patients admitted in the intensive care unit of a tertiary care centre. Strain of *P. aeruginosa* resistant to imipenem, were tested for metallo beta-lactamase production by phenotypic methods. **Result**: Out of 103 Pseudomonas aeruginosa, 48 strains were found imipenem resistant, of which 43(89.6%) were MBL positive by imipenem -EDTA combined disc test and the rest 35(72.9%) were by modified Hodge test. **Conclusion**: Imipenem – EDTA combined disc test found to be the best phenotypic method for detection of MBL. The appearance of the MBL and their spread among bacterial pathogens is a matter of concern with regard to the future of antimicrobial therapy. Both the methods are simple and one of these phenotypic methods can be easily incorporated in routine lab procedures to detect MBL.

Key words: *metallo beta-lactamases, Imipenem– EDTA combined disc test, Modified Hodge Test.*

Pseudomonas aeruginosa is one of the leading causes of nosocomial infections and due to its numerous intrinsic and acquired mechanisms of drug resistance, it is a cause of concern for treating clinicians. Although the antibiotic resistance in *P.aeruginosa* is caused by multiple mechanisms, one growing factor leading to resistance is the production of carbapenemases [1]. Carbapenemases are the acquired Metallo-β-lactamases (MBLs) which require zinc at the active site. They belong to Ambler's Class B and Bush-Jacoby Mederios group 3. In India, bla Verona imipenemase (VIM) and NDM-1 have been reported in *P.aeruginosa* [1]. *Pseudomonas aeruginosa* producing MBLs was first reported from Japan in 1991 and since then these have been described from various parts of the world including Asia, Europe, Australia, South America and North America [2,3].

MBLs have the ability to hydrolyze a wide variety of beta lactam agents, such as penicillins, cephalosporins and carbapenems but not Monobactams [4]. MBLs can be divided into six categories according to their molecular structure namely- imipenem (IMP), VIM, German imipenemase (GIM), SIM, SPM and AIM. These enzymes require zinc for their catalytic activity and are inhibited by metal chelators, such EDTA and thiol compounds [5].

MBLs have recently emerged as one of the most problematic resistance mechanisms owing to their capacity to hydrolyze all beta lactams including carbapenems with exception of Aztreonam [6]. Acquired MBL genes are located on integron structures that reside on mobile genetic elements such as plasmids or transposons, thus enabling widespread dissemination [7]. Carbapenemases were formerly believed to be derived only from classes A, B, and D, but a class C carbapenemase (CMY) has been described. These enzymes fall into three of the Ambler classes of beta-lactamases A, B and D [8].

MBL producing *P.aeruginosa* isolates have been report¬ed to be important causes of nosocomial infections across the world. These constitute 20-42 per cent of all noso¬comial isolates [9]. Detection of carbapenemases is difficult. It can be detected by phenotypic as well as genotypic methods [10]. Among phenotypic tests, MHT is a relatively easy and simple test to be performed in a laboratory. MHT is as the name says is the modified version of Hodge test which was used some years ago [10,11]. Even the original Hodge test was evaluated utilizing the PCR confirmed IMP-1 and VIM-2 MBL producing isolates. The original Hodge test utilized imipenem 10µg disc which gave fairly good results as it detected 67% cases of MBL producing *Pseudomonas aeruginosa* and Acinetobacter species [11]. The present study was undertaken to detect the prevalence of MBL in clinical strains of *Pseudomonas aeruginosa* isolated from ICU of a tertiary care centre Bareilly, UP and to evaluate the accuracy of two phenotypic tests combined disc test & modified Hodge Test for the detection of MBLs in *P. aeruginosa* isolates.

METHODS

A prospective study was conducted over a period of 18 months (January 2013 to June 2014) at the Department of Microbiology, in a tertiary care centre in Bareilly. This study was approved by the institutional ethical committee members and a total of 103 *Pseudomonas aeruginosa* were isolated from 243 patients. The patients and other concerned persons were made aware of the study and were signed for their consent form. The sputum, broncho alveolar lavage (BAL), pus, urine, blood samples from critically ill patients admitted in the intensive care unit of a tertiary care centre.

Specimens were inoculated on MacConkey agar, CLED, blood agar and blood culture bottle according to sample received in laboratory. The culture plates were aerobicaly incubated at 37°C for 18-24hrs followed by observation of colony characteristics, cell morphology by grams staining, and biochemical reaction, *pseudomonas aeruginosa* were confirmed as per standard protocol.

Antimicrobial sensitivity was performed on Mueller Hinton agar plates (Hi-Media, Mumbai) by Kirby-Bauer disk diffusion method according to Clinical & Laboratory Standards Institute (CLSI) full guidelines 2011. The antibiotic disks (Hi media, Mumbai, India) which were used are Cefepime (30µg) Ceftazidime (30 µg) Levofloxacin (15 µg), Amikacin (30), Meropenem(10 µg), Imipenem (10 µg) Cefoperazone/sulbactam (75/30µg) Piperacillin / Tazobactam (100/10 µg), Colistin (10 µg) Polymyxin(300 µg). The zone of inhibition was deter¬mined by measuring the disc diameter, after incubation of 24 hours at 37°C. P. aeruginosa ATCC 27853 was used as control. Isolates were considered to be imipenem resistant when the zone around imipenem was ≤ 13 mm, intermediate 14-15 mm and sensitive ≥16 mm. Among 103 *P. aeruginosa* isolated 48 isolates were imipenem resistant. All 48 Imipenem resistant Pseudomonas aeruginosa were screened for MBL production by Combined-disk method & Modified Hodge test.

In Imipenem (IMP)-EDTA combined disc test, the test organisms were inoculated by lawn culture technique on the plates of MHA as recommended by CLSI. Imipenem discs of 10 µg and 750 µg Imipenem-EDTA Disk (Hi-media SD281) were placed on the plate. The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 16 to 18 hours of incubation at 37°C. In the combined disc test, if the increase in inhibition zone with the imipenem and EDTA disc was found to be ≥ 7 mm than the imipenem disc alone, it was considered as MBL positive.

MHT detects carbepenemase production in gram negative isolates. An overnight culture suspension of Escherichia coli ATCC 25922 adjusted to 0.5 McFarland, was inoculated using a sterile cotton swab on the surface of MHA. After drying, 10 µg imipenem discs was kept at the centre of the MHA plate and the test strains suspension was inoculated by streaking method from the edge of the imipenem disc to the periphery of the petri-plate in four different directions followed by incubating the plates at 37°C overnight. . When the plates were observed, if the test strain that are carbenemase producing, had a presence of “cloverleaf shaped” zone of inhibition, then the test organism was considered as MBL positive.

RESULTS

In this prospective study where, 103 *Pseudomonas aeruginosa* stains obtained from the patients, 48 imipenem resistant strains of Pseudomonas aeruginosa were subjected to MBL detection by two phenotypic methods. After all the two staines were detected by two methods separately i.e. imipenum-EDTA combined disc test and MHT we found 43 (89.6%) MBL producer by Imipenem-

EDTA combined disc test & 35 (72.9%) by MHT as shown in table 1.

Table 1 - Comparison of Imipenem-EDTA Combined Disc Test and Modified Hodge Test

Method	Total P. aeruginosa	Imipenem Resistant	MBL Positive	%
Imipenem-EDTA Combined Disc Test	103	48	43	89.6%
Modified Hodge Test	103	48	35	72.9%
Inference	Both are significantly correlated (p = 0.036)			

DISCUSSION

Today, *Pseudomonas aeruginosa* is considered as a global threatening nosocomial infection and this have emerged as a trouble maker for the medical fraternity. Their remarkable growth in developing resistance to most antibiotic classes, including carbapenems, gives them a lot of importance. Hence, there is an emerging need for identification of the MBL and other mechanisms of drug resistance, else we may soon be facing end of the antibiotic era. These isolates also showed significant co-resistance to other class of antibiotics thus leaving behind only few therapeutic options. Therefore these β-lactamases should be detected routinely in clinical laboratories by using appropriate methods and reported to clinicians at times so that inappropriate use of antibiotics can be stopped on time.

Reportedly, there has been prevalence of carbapenem resistance mediated by obtained MBLs, particularly for *Pseudomonas aeruginosa* clinical isolates in several countries [12]. To prevent the necessary spread of MBL producing gram negative bacteria; rapid detection of MBL is required. One such method was performed in a study by Gibb et al. [12], where, using a disk with imipenem plus 750 µg of EDTA differentiated all MBL-producing pseudomonads. Carbapenems have a broad spectrum of antibacterial activity against most aerobic and anaerobic Gram-positive and Gram-negative organisms.

Carbapenems, if not equal, are slightly more active than, imipenem against Gram-negative aerobic bacteria and slightly less active than imipenem against Gram-positive organisms. It is stable to hydrolysis by dehydropeptidase. It is not hydrolyzed by most serine β-lactamases, but like all carbapenems and penems is readily hydrolyzed by carbapenemases. Hence, they are often used as a last resort in treatment. These are resistant to hydrolysis by most β-lactamases including extended spectrum β-lactamases (ESBL) and AmpC β- lactamases.

There has been an increase in reports of Carbapenem resistance in *P. aeruginosa* worldwide. In India, the first report of MBL was published from Bangalore and they reported 12% of the isolates which were resistant to both beta lactamase inhibitors and Carbapenem and 100% of these isolates were found to be MBL producer [13]. Whereas, our study shows correlation in terms of the first method i.e. the Imipenem-EDTA combined disc test where, almost 90% of the study population was found to be MBL producer.

In India, a lot of studies have been done on MBT producing non fermenters and the results vary all over the country. It has been reported as low as 7.5% to as high as 100% [13]. In the study done by Navaneeth BV et. al. in 2002 found the prevalence of MBL 15/68 (22.05%) among carbapenem resistant isolates. These findings were not similar to the present study. The isolate were resistant to either or both Imipenem and Meropenem. The criteria for choosing the isolates for MBL screening are varied. Some studies have chosen Ceftazidime resistant strains for screening MBL While most of the studies have chosen Imipenem resistant strains for screening of MBL [14-17]. In our present study, 43 (89.6%) isolates of *Pseudomonas aeruginosa* were positive for Metallo β lactamases production by method-1 and 35 (72.9%) were positive by method-2.

This study results demonstrate the serious therapeutic and epidemiological threat of the spread of MBL producing *Pseudomonas aeruginosa*. In this study, Imipenem-EDTA combined disc test was found to be best screening tests followed by MHT. This study has a limitation of sampling, since all the samples were taken from a single hospital and a study with patients varying in locations, is suggested to get more précised and accurate results.

CONCLUSION

The β-lactamases should be detected routinely in clinical laboratories by using appropriate methods and reported to clinicians at times so that inappropriate use of antibiotics can be stopped on time. Excellent antimicrobial policies and infection control implementation are important priorities for the critical patient areas.

REFERENCES

1. S Buchunde, DK mendriatta, VDeotale and P Narang. Comparison of disc and MIC reduction with polymerase chain reaction for detection of metallo-β-lactamase in

Pseudomonas aeruginosa. Indian J Med Mirobiol 2012; 30(2):170-174.
2. Wantanabe M, Iyobe M, Mitsuhashi S. Transferable Imipenem resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 1991; 35:147-51.
3. Saderi H, Karimi Z, Owlia P, Bahar MA, Bagher SM. Phenotypic detection of metallo-beta-lactamase producing Psuedomonas aeruginosa strains isolated from burned patients. Iran J Path 2008; 3(1): 20-4.
4. Pitout JDD, Revathi G, Chow BL, Kabera B, Kairuki S, Nord¬mann P, et al. Metallo-β-lactamase –producing Pseudomonas aeruginosa isolated from a tertiary care centre in Kenya. Clin Mi¬crobiol Infect 2008; 14(8):755-9.
5. Livermore DM, Woodford N. Carbapenemases: a problem in waiting? Curr Opin Microbiol 2000; 3:489-95.
6. B Behera,P Mathur ,A Das,A Kapil and V Sharma.An evaluation of four different phenotypic techniques for detection of metallobetalactamase produing Pseudomonas aeruginosa.Indian J Med Microbiol 2012; 26(3):233-37.
7. Shobha KL, Lenka PR, Sharma MK, Ramahandra LBI. Metallobetalactamase production among Pseudomonas species and Acinetobacter species in coastal Karnataka. J Clin Diagn Res 2009; Oct13(3):1747-1753.
8. Carbapenemases Beta-lactamase-Wikipedia, the free encyclopedia. http;//en.wikipedia.org/wiki/Beta-lactamase. Last accessed on 20th July 2018.
9. Tenover C F. Mechanisms of Antimicrobial Resistance in Bacteria. AM J Med 2006; 119(6A) [PubMed].
10. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-b-lactamase: the Quiet before the Storm? ClinMicrobiol Rev 2005; 18:306-25.
11. Gibb AP, Tribuddharat C, Moore RA, Louie TJ, Krulicki W, Livermore DM, et al. Nosocomial outbreak of carbapenem-resistant Pseudomonas aeruginosa with a new blaIMP allele, blaIMP-7. Antimicrob. Agents Chemother 2002; 46:255-258.
12. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-Lactamases producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2002; 40:3798-801.
13. Amjad A, Mirja IA, Abbasi SA, Farwa U, Malik N, Zia F. Modified Hodge Test: A simple and effective test for detection of carbepenemase production. Iran. J. Microbiol. 2011; 3(4): 189-193.
14. Navaneeth BV, Sridaran D, Sahay D, Belwadi MR. A preliminary study on metallo-[beta]-lactamase producing Pseudomonas aeruginosa in hospitalized patients. Indian Journal of Medical Research, 2002; 116:264.
15. Hemalatha V, Sekar U, Kamat V. Detection of metallo betalactamase producing Pseudomonas aeruginosa in hospitalized patients. Indian Journal of Medical Research 2005; 122(2):148.
16. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-β-lactamase-producing gram-negative bacteria by using thiol compounds. Journal of Clinical Microbiology 2000;38(1):40-43.
17. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem-susceptible metallo-β-lactamase-producing gram-negative bacilli in the clinical laboratory. Journal of clinical microbiology 2006; 44(9):3139-44.

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